STUDY OF COD REDUCTION BY ANAEROBIC DIGESTION USING MIXED CULTURE FROM INDUSTRIAL WASTE

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ABSTRACT

The study of COD reduction using mixed culture from paper mill wastewater has been investigated. The treatment is using anaerobic digestion. Two Litre Scott bottle was used as digester. The gas production from the digestion process was also studied. The methodology consists of 2 main parts. The first part is the culturing procedure, in which the mixed culture was prepared by culturing the microorganisms from the wastewater sample that was collected from the treatment plant of Pascorp Paper Industries in Bentong, Pahang. The second part is treatment of synthetic waste using anaerobic digestion. Four digesters with glucose concentrations of 1 to 4 g/L were prepared. The digestion process was initiated by mixing the mixed culture with the glucose. The digesters were closed and the experiment was run for 5 days. Sample was taken every day from each digester to check the COD value and turbidity. The gas production rate was also measured using the water displacement unit. The results show that COD reduction of over 90% was achieved after 5 days of digestion in all concentrations of glucose. The gas production rate was not consistent with respect to the glucose concentration and time of digestion but the average gas production rate shows that the higher the glucose concentration the higher the gas production. In conclusion, the mixed culture collected from wastewater of paper mill has proved its ability to efficiently reduce the COD concentration. The average gas production rate shows a higher rate at digestion of higher glucose concentration.

ABSTRAK

Penyelidikan berkenaan penurunan COD menggunakan kultur campuran dari air sisa kilang kertas telah dikaji. Rawatan ini menggunakan kaedah pencernaan anaerobik. Botol Scott dengan isipadu dua liter digunakan sebagai pencerna. Selain itu, penghasilan gas melalui proses pencernaan tersebut turut dikaji. Metodologi kajian ini terdiri daripada dua bahagian utama. Bahagian pertama adalah prosedur pengkulturan, di mana kultur campuran disediakan dengan mengkulturkan mikroorganisma dari sampel air sisa yang dikumpul dari loji rawatan Pascorp Paper Industries di Bentong, Pahang. Bahagian kedua adalah rawatan air sisa tiruan dengan menggunakan kaedah percernaan anaerobik. Empat pencerna dengan kepekatan glukosa 1 hingga 4 g/L disediakan. Proses pencernaan dimulakan dengan mencampur kultur campuran dengan glukosa tersebut. Kesemua pencerna ditutup dan eksperimen dijalankan selama 5 hari. Setiap hari, sampel diambil dari setiap pencerna untuk dianalisis nilai COD dan kekeruhan. Kadar penghasilan gas juga diukur melalui unit sesaran air. Keputusan kajian menunjukkan bahawa penurunan COD melebihi 90 % selepas 5 hari proses pencernaan bagi setiap kepekatan glukosa. Kadar penghasilan gas adalah tidak konsisten dengan kepekatan glukosa dan tempoh pencernaan tetapi secara purata, kadar penghasilan gas menunjukkan bahawa semakin tinggi kepekatan glukosa penghasilan gas turut meningkat. Kesimpulannya, kultur campuran yang dikumpulkan dari air sisa kilang kertas telah membuktikan kemampuannya untuk mengurangkan kepekatan COD dengan efisien. Penghasilan gas secara puratanya menunjukkan tahap pencernaan lebih tinggi pada kepekatan glukosa yang lebih tinggi.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Wherever there is human settlement, there is a need to treat waste generated from a great deal of human activity. Along with the advancement of human activity and standard of life, the waste content from various sources of human activities are also getting more and more complicated and difficult to be degraded. Therefore wastewater treatment has become one of the more important fields of engineering to minimize its impact on environment.

Domestic and industrial wastewater typically contains very high portion of substances that are harmful to the environment if not well treated. The amount of pollutants that exist in certain waste and their impact to the environment are dependent on the sources of the wastes. Wastewater from different sources have different constituent, hence they have to be treated accordingly before it can be discharged. The major contaminants found in wastewater are biodegradable organic compounds, volatile organic compounds, recalcitrant xenobiotics, toxic metals, suspended solids, nutrients (nitrogen and phosphorus), and microbial pathogens and parasites. Efforts are now being focused on the removal of nutrients, odour, volatile

organic compounds, metals and toxic organics. The principle objectives of waste treatment processes as described by Bitton (2005) are:

1. Reduction of the organic content of wastewater (reduction of biochemical oxygen demand, BOD).
2. Removal/reduction of trace organics that are recalcitrant to biodegradation and may be toxic or carcinogenic.
3. Removal/reduction of toxic metals.

It has become enormously important to direct research efforts toward sustainable methods that not only alleviate environmental pollution, but also ease the stress on depleted natural resources and growing energy insecurity. The most cost-effective and sustainable approach is through the biological treatment. Aerobic processes are widely used worldwide for municipal wastewater treatment and nowadays the anaerobic approach has become increasingly important (Khanal, 2008).

Anaerobic digestion is an effective way to treat wastewater and its popularity has grown over decades in many countries. Completely mixed anaerobic digesters are the most commonly used treatment system in North America for the degradation of municipal sludges, and there are increasing number of industrial plants are using fixed-film anaerobic digesters for the treatment of soluble organic compounds in their wastewaters (Gerardi, 2003).

From the perspertive of developing and underdeveloped countries, a wider application of anaerobic digestion technology has even greater impacts, due to the fulfillment of three basic needs: (a) Improvement in health and sanitation through pollution control; (b) Generation of renewable energy for household activities and (c) supply of digested materials (biosolids) as a biofertilizer for crop production. The significance of anaerobic digestion technology is not only in controlling pollution but also in supplementing valuable resources in terms of energy and value-added products (Khanal, 2008).

Legislation is now in place which demands high standard of effluent treatment and waste disposal, both industrial and domestic. Formidable legislation is being slowly enacted in Europe, the United States and the United Kingdom, that demands high standards of effluent treatment and waste disposal (Arundel, 2000). Malaysia being one of the leading developing countries is of course not far behind in applying strict legislation and new technology to control the quality of waste effluent. Anaerobic digestion has certainly become one of the more cost effective choices of wastewater treatment process. Thus the study of wastewater treatment using anaerobic digestion process is crucial to increase its efficiency as well as widen its application towards the various types of wastes generated in Malaysia.

1.2 Problem Statement

The economics of the process is substantially depending on the efficiency of the wastewater treatment process. Chemical oxygen demand (COD) is one of the primary parameters that indicate the level of pollution. Conventional wastewater treatment plant is often bothered by the high cost and high process complexity. Utilizing anaerobic digestion in treating wastewater has proved to be successful in degrading organic waste and metal ions from household and industry. However, the efficiency of the anaerobic digestion process is governed by the type of microorganisms that present and the type of waste component that they can digest, along with the condition of process and the time needed for effective digestion so that desired level of COD reduction can be achieved. The effects of different factors towards reduction of COD in the anaerobic digestion process need to be investigated. Also, a high energy input is required for conventional wastewater treatment process. The investigation on the biogas produced from anaerobic digestion could become a promising source of renewable energy.

1.3 Research Objectives

1. To determine the percentage of chemical oxygen demand reduction in different glucose concentration.
2. To determine the amount of biogas that is produced throughout the anaerobic digestion process.

1.4 Scope of Research

The research will be conducted in 2 L Scott bottles as digesters. Glucose is used as the synthetic waste for this study to better predict the results and to avoid the results being affected by nature of real waste which contains impurities, debris and toxic substances. Four digesters that contain glucose concentrations from 1 g/L to 4 g/L will be set up. The process is anaerobic digestion and the Schott bottles will be sealed to avoid exposure to oxygen. The biogas produced will be collected using the water displacement method. Variable to be controlled is the concentration of glucose, while the parameters that to be studied are the chemical oxygen demand, turbidity and the gas production rate. The mixed culture used is originated from paper mill wastewater and the sample is collected from Pascorp Papers Industries in Bentong, Pahang. The digestion process will be run for 5 days.

CHAPTER 2

LITERATURE REVIEW

2.1 Anaerobic Digestion

Kalyuzhnyi & Davlyatshina (1997) defined anaerobic digestion as a complex multistage process of organic compound degradation to methane and carbon dioxide by the action of numerous anaerobic microflora. While according to Khanal (2008), anaerobic processes can be defined as biological processes in which organic matter is metablolized in an environment free of dissolved oxygen or its precursors. It is classified as either anaerobic fermentation or anaerobic respiration, depending on the type of electron donor. The anaerobic digestion is by far the most common process to treat waste-water sludges containing primary sludge. Primary sludge contains numerous readily available organics that would induce a rapid growth of biomass if treated aerobically. Anaerobic decomposition produces considerably less biomass than aerobic process. The principal function of anaerobic digestion is to convert as much of the sludge as possible to end products such as liquids and gases, while producing as little residual biomass as possible (Peavy *et. al.*, 1985).

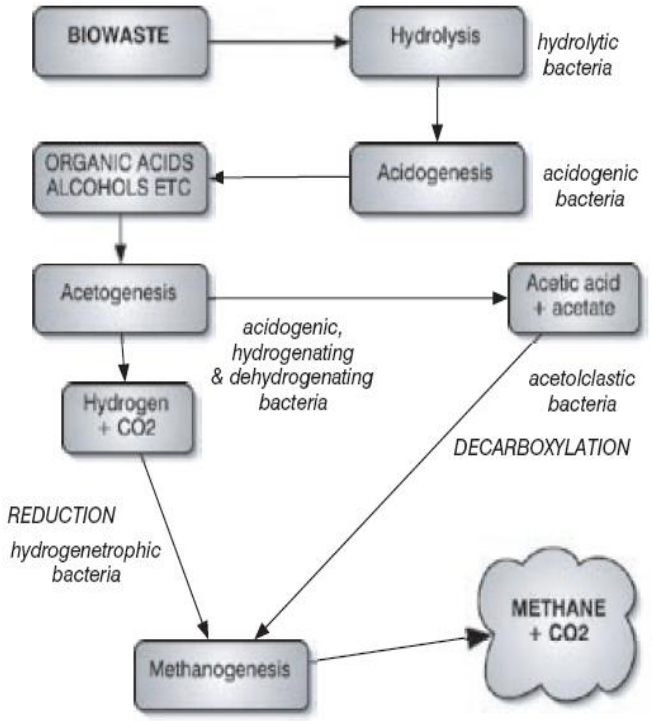

Figure 2.1: Flowchart indicating the main stages in anaerobic digestion

Figure 2.1 shows the principle processes involved in the anaerobic digestion, which consists of mainly 3 stages: acidogenesis, acetogenesis and methanogenesis. As many wastes can be treated anaerobically, the feasibility of anaerobic digestion of waste is determined by several factors. Khanal (2009) stated that temperature, operating pH, oxidation-reduction potentials and nutrients or trace metals are the main factors that may affect the anaerobic digestion process. Other factors include the concentration of the waste, the temperature of the waste stream, the presence of toxicants, biogas and sludge production, and expected treatment efficiency (Gerardi, 2003). The comparison between anaerobic digestion and aerobic digestion is shown in Table 2.1.

Table 2.1: Comparison between Anaerobic Digestion and Composting (Aerobic Digestion)

Features	**Anaerobic Digestion**	**Composting**
Space requirement (footprint)	50%	100%
Odours	20%	100%
Energy balance	Energy surplus	Energy demand
Biogas production	100 – 150 m^3/mg	Nil
Process time required to produce mature compost	3 weeks digestion, plus 5 weeks composting	12 weeks

(Muller & Huttner, 2005)

2.2 Process Microbiology

Bacteria and methanogens are the dominant microorganism in anaerobic digesters, though some fungi and protozoa may also be found. As described by Bitton (2005), there are four categories of microorganisms involved in the transformation of complex materials into simple molecules such as methane and carbon dioxide. They operate in a synergistic relationship.

The first group is the hydrolytic bacteria. The consortia of anaerobic bacteria break down complex organic molecules such as cellulose and lignin into soluble monomers like amino acids, glucose, fatty acids and glycerol, so that they are available for the next group of bacteria, which are the fermentative acidogenic bacteria. Example of these acid-forming bacteria is *Clostridium*, which converts sugar, amino acids and fatty acids to organic acids, alcohols and ketones, acetate,

carbon dioxide and hydrogen. The products formed vary with the bacterial type and the culture conditions such as temperature, pH and redox potential (Bitton, 2005).

The third group of microorganism is the acetogenic bacteria. Examples of these acetate and hydrogen-producing bacteria are *Syntrobacter wolinii* and *Syntrophomonas wolfei*. They convert fatty acids (e.g. propionic acid, butyric acid) and alcohol into acetate, hydrogen and carbon dioxide. Low hydrogen tension is required for the conversion, as under relatively high hydrogen partial pressure, acetate formation is inhibited and the substrate is converted to propionic acid, butyric acid and ethanol rather than methane (Bitton, 2005).

The final group of microorganisms is the methanogens. Methanogenic microorganisms grow slowly in wastewater and their generation times range from 3 days at 35ºC to as high as 50 days at 10 ºC. There are two subcategories of methanogens. The hydrogenotrophic methanogens convert hydrogen and carbon dioxide into methane, while the acetotrophic methanogens (genera *Methanosarcina* and *Methanothrix* and *Methanosaeta*) convert acetate into methane and carbon dioxide. About two-thirds of methane is derived from acetate conversion by acetotrophic methanogens. (Bitton, 2005)

2.3 Mixed culture

A mixed culture is a microbial culture that contains two or more different strains of organisms. The use of mixed culture provides several advantages over a pure culture. The mixed culture can better adapt to changing conditions during growth (Nor Habibah, 2006). According to Bailey and Ollis (1986), natural occurring mixed cultures are particularly efficient means for utilization of substrate mixtures in the context of wastewater treatment. Works by Sineriz and Pirt (1977) has shown the

mixed culture that is yielded from formate-limited growth contains predominantly methanogenic bacteria morphologically similar to *Methanobacterium formicicum* and *Methanobacterium ruminantium*. A facultative anaerobe capable of metabolizing glucose known as *Citrobacter* has yielded acetate, formate, ethanol and lactate as its products, and the lactate produced can be used as substrate to form methane by other methanogens. This shows that the mixed culture provides more alternatives or process pathway for the formation of anaerobic digestion to produce methane and carbon dioxide.

2.4 Wastewater from Paper and Pulp Industry

Works by Rajeshwari *et. al.* (1999) on treatment of paper and pulp wastewater has shown that a four stage treatment process that consist of pre-treatment, anaerobic treatment using an UASB, aerobic treatment and tertiary flotation was found to be successful in reducing the COD. The anaerobic treatment of paper and pulp wastewater must combine with other pre-treatment as the effluent contained refractory compounds such as lignin derivatives, resins and tannins apart from sugar. COD reduction of 80% and 0.34 m^3/kg COD removed were achieved for anaerobic treatment of high strength effluent from acid hydrolysis. Hence it shows that the wastewater from paper and pulp industry which involved in anaerobic treatment can be used as a source of methanogens.

2.5 Chemical Oxygen Demand

The chemical oxygen demand (COD) test is widely used as a means of measuring the organic strength of domestic and industrial wastes. The COD test uses

a strong chemical oxidant in an acid solution and heat to oxidize organic carbon to CO_2 and H_2O. By definition, chemical oxygen demand is "a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant." Oxygen demand is determined by measuring the amount of oxidant consumed using titrimetric or photometric methods. The test is not adversely affected by toxic substances, and test data is available in 1-1/2 to 3 hours, providing faster water quality assessment and process control. Measurement of nonbiodegradable organics is usually by the COD test. (Peavy *et. al.*, 1985)

One of the main limitations of the COD test is its inability to differentiate between biologically oxidizable and biologically inert organic matter. In addition it does not provide any evidence of the rate at which the biologically active material would be stabilized under conditions that exist in nature. (Sawyer *et. al.*, 2005)

2.6 Kinetic Study of Anaerobic Digestion of Glucose

The kinetic study of batch anaerobic digestion of glucose (Table 2.2, initial pH 7.0) from Kalyuzhnyi & Davlyatshina in 1997 showed that, besides methane and carbon dioxide as the main final products, some intermediate byproducts (ethanol, acetate, propionate, butyrate and hydrogen), concentrations of which passed through maxima, were also detected in the reactor medium. This reflects the multistep nature of glucose anaerobic digestion which is believed to include three main steps: acidogenesis, acetogenesis and methanogenesis. During the acidogenesis, the following step equation applies:

$$C_6H_{12}O_6 \rightarrow 1.14H_2 + 1.14CO_2 + 0.34C_2H_5OH + 1.31CH_3COOH + 0.19C_3H_7COOH + 0.2\,C_2H_5COOH \quad (1)$$

During anaerobic digestion of intermediates of glucose decomposition, ethanol, propionate and butyrate are first converted into acetate and hydrogen before undergoing methanogenic step. The following equations describe the chemical conversion in the acetogenic step:

$$C_2H_5OH + H_2O \rightarrow CH_3COOH + 2H_2 \quad (2)$$

$$C_3H_7COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2 \quad (3)$$

$$C_2H_5COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 2H_2 \quad (4)$$

The reactions for methanogenic step are as followed:

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (5)$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (6)$$

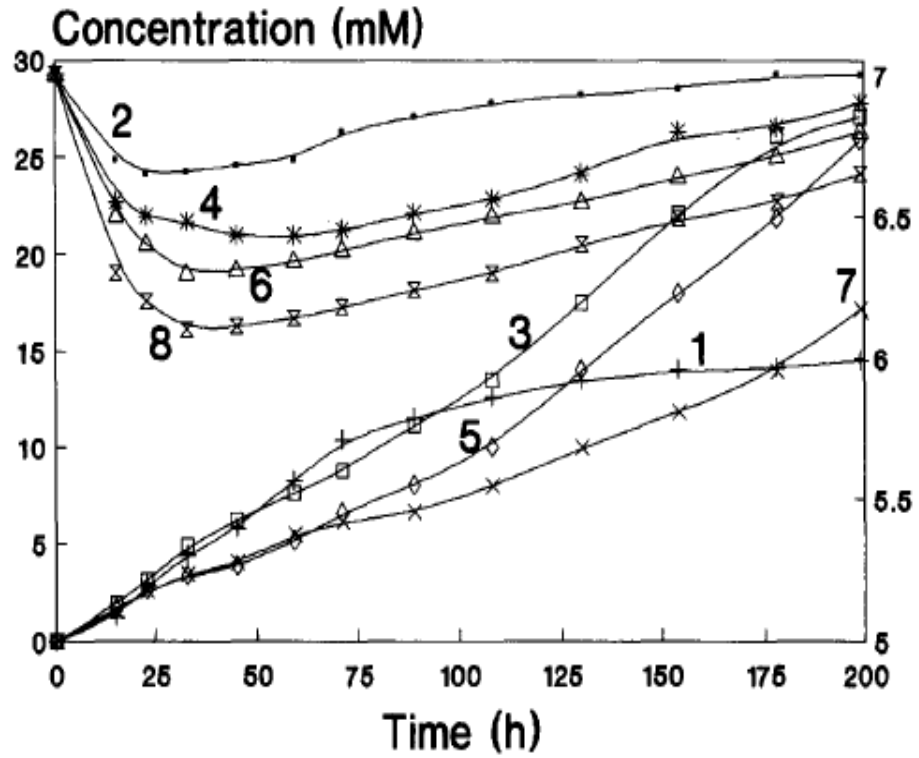

Figure 2.2: Kinetics of methane formation and pH under variation of initial glucose concentration (1,3,5 and 7 refers to methane while the rest are pH)

2.7 Review of Other Related Studies

Related studies on anaerobic digestion of organic waste and the main findings are presented in Table 2.3.

Table 2.2: Review of related studies

Author	**Title of Journal**	**Findings/Desciption**
G. Lettinga (1994)	Anaerobic digestion and wastewater treatment systems	• The UASB-process can be applied at high space loading rates (> 25 kg COD/m^3.day) for very different industrial effluents varying in COD from approximately1.5 kg/m^3 to over 100 kg/m^3.
Zhi Li, Hui Wang, Zongxun Tang, Xiaofang Wang, Jinbo Bai (2008)	Effects of pH Value And Substrate Concentration on Hydrogen Production from the Anaerobic Fermentation of Glucose	• Optimal condition for anaerobic fermentative hydrogen production is 7.5 g glucose/L and constant pH 6.0 • maximum H_2 production rate of 0.22 mol H_2 mol^{-1} glucose h^{-1} • a cumulative H_2 yield of 1.83 mol H_2 mol^{-1} glucose and a H_2 percentage of 63 in biogas.
S. V. Kalyuzhnyi & M. A. Davlyatshin (1997)	Batch Anaerobic Digestion Of Glucose And Its Mathematical Modeling. I. Kinetic Investigations	• No noticeable inhibition of methane production up to the initial glucose concentration of 11 mM. • Further increase of the initial glucose concentration caused a decrease of the methane formation rate in the time range of 100 h

Yoshiyuki Ueno et al. (2006)	Production Of Hydrogen and Methane From Organic Solid Wastes by Phase-Separation of Anaerobic Process	• packed-bed reactor • produced approximately 442mmol^{-1}days^{-1} of methane • 199 mmol^{-1} days of hydrogen at 25 h of total retention time • 82% of COD removal
Nor Habibah Binti Mohd Rosli (2006)	Development of Biological Treatment System For Reduction of COD From Textile Wastewater	• 60% of COD removal using single culture of microorganism • 90% of COD removal using mixed culture • Lower biomass concentration resulted in higher COD reduction using freeze-dried culture • Faster treatment time (2 days) was also achieved using freeze-dried culture compared to liquid culture (5 days)

CHAPTER 3

METHODOLOGY

3.1 Introduction

The general idea of the project is to study the COD reduction of different concentration of glucose when mixed culture is added. The other parameters to be studied include the turbidity and gases production.

Schott bottles with a capacity of two litres were used as digester for the digestion process to be carried out. The Schott bottles caps were modified so that to allow tubes to be attached for gas collection and also to allow sample collection for COD and turbidity reading. Prior to the setup of experiment, the mixed populations of microorganisms that were retrieved from the paper mill wastewater were being cultured in the laboratory to obtain higher amount of microorganisms.

Hence the whole methodology can be divided into three parts. The first part is the collection of industrial wastewater sample from the industry. The second part is the culturing of microorganisms in the mixed culture and the third part is the setting up and running of experiment.

Figure 3.1 shows the general methodology in conducting this project and the major stages involved.

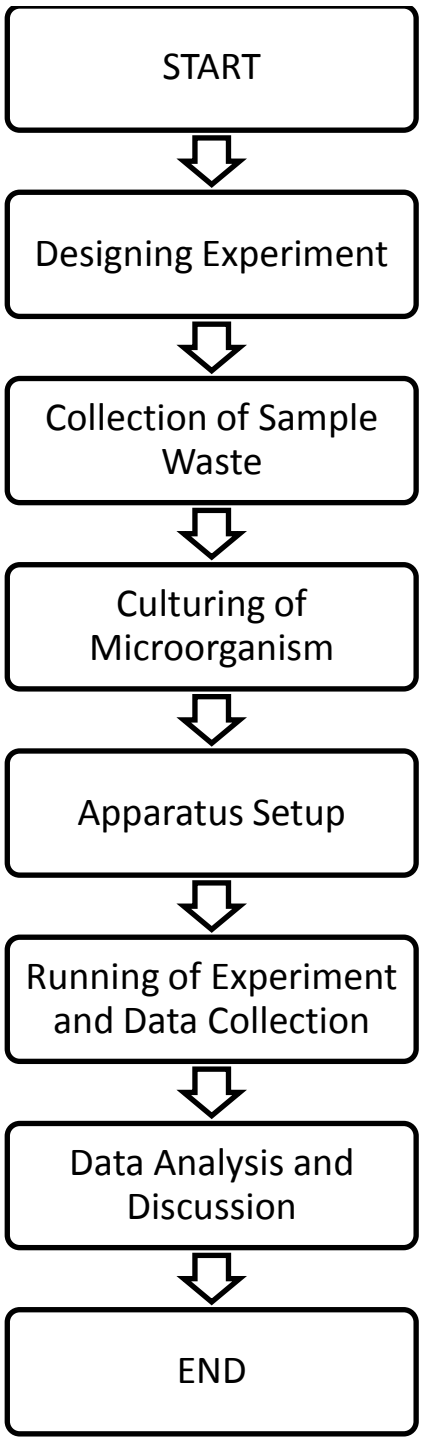

Figure 3.1: The overall Methodology

3.2 Materials and Apparatus

The materials and apparatus used are dependent on the procedure involved in different parts of the methodology. There are generally three main parts of methodology and each involves different procedure.

For collection of sample waste, the required equipments are Schott bottles, gloves, funnel, ethanol sprayer as disinfectant and refrigerator to keep the sample. For culturing of microorganism, the main materials and equipments are nutrient broth, agar plates, glucose stock and inoculation apparatus. The instruments used are autoclave, laminar flow chamber and shaker incubator. As for the main experiment, the materials and equipments to be prepared are glucose, COD reagent vials (high range), Schott Bottles 2L, tubes, retort stands, measuring cylinders 250ml, syringe, the HACH DR/2400 @ DR/2800, turbidity meter and stop watch.

3.2.1 Source of Mixed Culture

The mixed culture was originated from the paper and pulp industrial wastewater. The wastewater sample was taken from Pascorp Paper Industries in Bentong, Pahang, which is a paper mill. The paper mill has an extensive wastewater treatment plant which involves biological means. The same wastewater was retrieved from the plant and will be used as the source of the mixed culture to treat the glucose.

3.3 Collection of Sample

The wastewater sample was contained in Schott bottle. Two 250 ml Schott bottles were used as container and were autoclaved beforehand to prevent contamination to the sample. Ethanol 70% was prepared to disinfect the apparatus for collection. Another 2 L Schott bottle was also prepare to take more samples for other uses.

The bucket and funnel were disinfected by spraying with ethanol. After the ethanol was dried out, the wastewater was filled into the sterilized Schott bottles. To reduce the risk of contamination, the Schott bottles were only opened as soon as the wastewater was ready to be filled and closed immediately after they were being filled. The collected samples were labeled and kept in the refrigerator while waiting to be transferred and cultured.

3.4 Culturing of the Microorganism

The aseptic technique, which is the series of steps used to prevent contamination during manipulations of culture and sterile culture media was applied in the preparation of mixed culture. All the culturing techniques were carried out with reference from Brock Biology of Microorganisms (Madigan and Martinko, 2006) and Laboratory Exercises in Microbiology (Harley, 2005).